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Controls on Denitrification and Nitrous Oxide Flux in Created Wetlands

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CONTROLS ON DENITRIFICATION AND NITROUS OXIDE FLUX IN CREATED WETLANDS

By:

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ABSTRACT

Freshwater wetlands are frequently created or restored with the goal of replicating valuable ecosystem functions lost elsewhere. However, studies in created wetlands have demonstrated production of greenhouse gases (GHG) may be enhanced during early establishment, potentially counteracting desirable ecosystem services such as nutrient removal. In this research, we investigated the impact of hydrology and carbon addition on denitrification and fluxes of N_2O from two created wetlands that differ in antecedent land use and hydrology. Ten experimental zones were installed in both wetlands and compost (municipal leaf litter) was added to half of the zones as a soil amendment. Soil and ecosystem N_2O fluxes, potential denitrification rates, soil properties and nutrient concentration were measured during the growing season of 2016. There was high variability in weather conditions during the study period, with drought conditions during the summer growing season. Compost addition significantly increased potential denitrification at both sites, but overall rates were driven by precipitation and nutrient availability. Soil N_2O fluxes were highly variable and correlated with precipitation patterns and nutrient availability, but were not impacted by compost addition. The key role of precipitation and temperature in GHG fluxes in both wetlands, implies susceptibility to ongoing climate changes. When creating wetlands, the regulation of nutrients, carbon and hydrology should be taken into consideration to limit GHG production and maximize desirable ecosystem services such as denitrification.

1. INTRODUCTION

The ecosystem services associated with wetlands are more valuable per hectare than any other ecosystem on Earth (Costanza et al., 1997). However, human population growth and land cover change, primarily for agricultural expansion, have destroyed nearly 50% of the global wetland area in the last century (Mitsch & Gosselink, 2000). Currently, the excessive use of fertilizers, industrialization, waste, invasive species and climate change are contributing to impaired functionality of remaining wetlands (Mitsch & Gosselink, 2007). Thus, to compensate for both historical and present threats to wetlands (Zedler, 2003), creation and restoration of wetlands has increased in the past four decades in order to replicate important ecosystem functions and services (Zhang et al., 2010). However, replication of natural ecosystem functions is a slow (Edwards & Proffitt, 2003) and very complex process (Hunting & Geest, 2011) that often fails to rapidly achieve multiple ecosystem functions (Zedler & Kercher, 2005). The success of created wetlands is influenced by a number of environmental factors, including prior land use, invasive species, nutrient pollution and unintended hydrologic conditions (Zedler & Kercher, 2005).

Wetland ecosystem functions are the result of synergistic interactions between biotic (living organisms) and abiotic (i.e. water, temperature, soil properties, pH and hydroperiod) factors that develop through time and ultimately under natural conditions act in concert to define wetland biogeochemistry and functionality (Maul et al., 1999; Zedler & Kercher, 2005). Certain key abiotic factors play a fundamental role in wetland biogeochemistry, especially the hydrology (Schaafsma et al., 1999; Sirivedhin & Gray, 2006, Ballantine et al., 2011), carbon availability (Sutton-Grier et al., 2009; Del Grosso et al., 2000; Ballantine et al., 2011) and nitrogen availability

(Gejlsbjerg et al., 1998; Sirivedhin & Gray, 2006; Hernandez & Mitsch, 2007) that may be associated with the historical use of the land prior the creation of the wetland. These interacting factors directly and indirectly control microbial communities and soil biogeochemical processes, such as denitrification, that influences the recycling and removal of nutrients (Saunders & Kalff, 2001). Denitrification in wetlands is of concern (Schaafsma et al., 1999) because high concentrations of reactive nitrogen can modify soil chemistry (Smith et al., 1998), degrade water quality, reduce biological diversity (Morris, 1991) and cause eutrophication. Further, when denitrification is incomplete, as occurs under certain unfavorable conditions common in created wetlands, the greenhouse gas N_2O may be released (Knowles, 1982; Vilain et al., 2014). Many of the factors that control denitrification in wetlands are the same ones that lead to the failure of created wetland projects; however, we lack a clear understanding of how denitrification varies across created wetlands that vary in prior land use and hydrology, or how management strategies may influence in the production and release of N_2O.

This study aimed to understand the relationship between the environmental properties of two created wetlands that vary in hydrology and prior land use influenced by compost addition as a management technique and their synergistic effect on denitrification. Of particular interest on how the different environmental circumstances and management strategies in the wetlands influence denitrification rates and N_2O production. The findings of this study will enhance successful long-term development of desirable ecosystem functions in created wetlands.

1.1 Created Wetlands

The loss of 53% of natural wetlands in the United States (Dahl, 1990) led to creation of the "No-Net Loss" policy of the Clean Water Act in the 1990s. This policy prohibits damage or destruction of wetlands; if damage cannot be avoided, wetland area must be replaced by creating new wetlands or restoring existing wetlands in order to preserve overall functions and services (Zedler, 1996). Thus, efforts to manage physicochemical, biological and ecological features of created wetlands to match natural wetlands have increased in the last forty years, mostly due to a high demand for replicating some important ecosystem functions such as regulation of nutrient cycling and water filtration (Zhang et al., 2010).

Created wetlands, also known as constructed wetlands, are man-made ecosystems built with the goal of replicating the ecosystem functions of natural wetlands (Yoshitaka & Sirintornthep, 2013). Under the Clean Water Act, these wetlands are expected to develop key ecosystem function within five to ten years of creation. In natural wetlands, the interaction between biotic and abiotic factors that develops through time defines wetland function (Figure 1). This interaction is a very complex and slow process; thus, it can take more than two decades for a created wetland to develop ecosystem functions similar to a natural reference wetland (Edwards & Proffitt, 2003). Prior work has demonstrated that created wetlands can replicate certain natural ecosystem functions, for example regulation of nutrients (Schaafsma et al., 1999), but at the same time fail to achieve others, such as plant community diversity (Zedler, 2003). The limitation to development of multiple ecosystem functions in created wetlands is an issue in ecological restoration projects, because it increases their vulnerability to climate change and

pollution and lowers productivity and ecosystem development. Abiotic factors such as soil properties, nutrient availability and hydrologic conditions are fundamental factors leading to unfavorable conditions for the establishment of biotic structure and diversity (animal, plant and microorganisms) associated with created wetlands. Understanding these interactive drivers of ecosystem development is crucial for managing created wetlands so that they approach functional equivalence with natural wetlands (Maul et al., 1999).

The biogeochemical cycling of a wetland can provide a systematic approach to the interaction between the living organisms and their physical environment (i.e. soil, water, nutrient availability, temperature, pH) that ultimately will define wetland structure and functionality (Maul et al., 1999). Recent studies have shown that denitrification rates in created wetlands are low compared to natural ones and this response is correlated to the age of the wetland (Wolf et al., 2011). For this reason, the study of biogeochemical processes such as denitrification can offer valuable information regarding how created wetlands are approaching natural reference wetlands and what factors and management techniques may promote more successful wetland function.

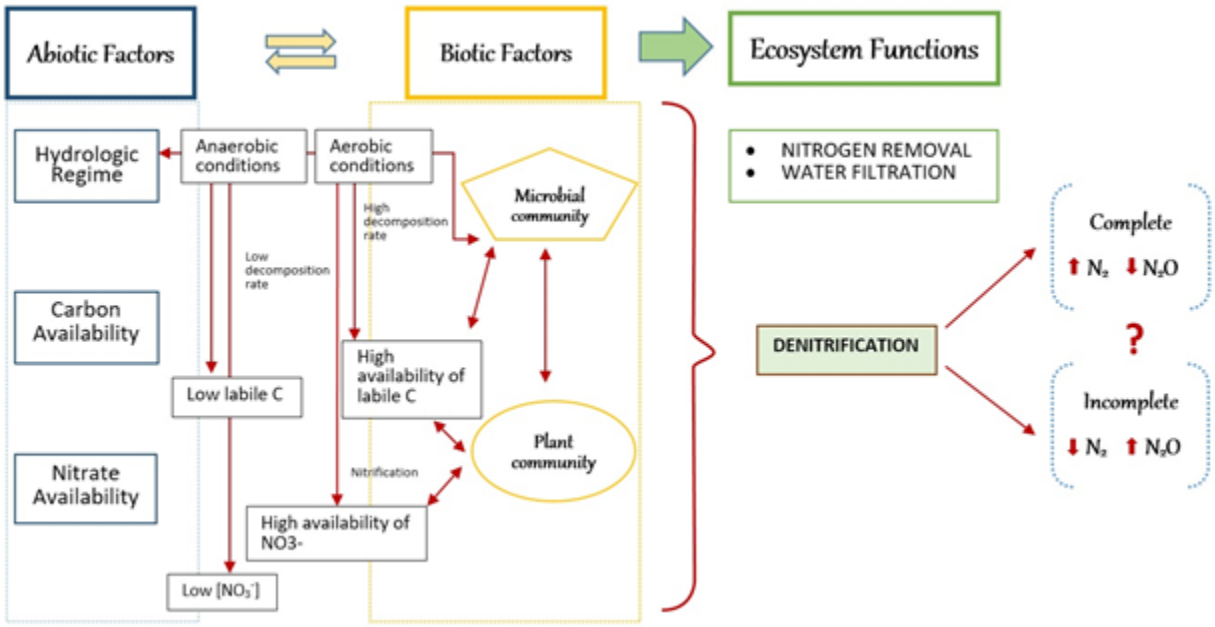

Figure 1. The relationship between biotic and abiotic factors and their impact on denitrification.

1.2 Nitrogen cycling in wetlands

In wetlands, the biogeochemical cycle of nitrogen is a series of processes mostly mediated by microbes that generally starts with nitrogen fixation. N fixation is the transformation of molecular nitrogen (N_2) to reactive nitrogen (NH_3) mediated by certain heterotrophic bacteria and cyanobacteria. Aerobic ammonification or mineralization processes where organic nitrogen is transformed to ammonium (NH_4^+) leads to "available" or "reactive" nitrogen. Nitrification, an aerobic process, is the oxidation of available ammonium to nitrate (NO_3^-). Although, under anaerobic conditions nitrate can be reduced to ammonium through dissimilatory nitrate reduction to ammonia (DNRA), or nitrite and ammonium can be reduced to nitrogen gas and water through anaerobic ammonium oxidation (ANAMMOX). Lastly, the process that completes the nitrogen cycle is denitrification, an anaerobic process whereby reactive nitrogen (nitrate and/or nitrite) returns to the atmosphere as molecular nitrogen (N_2) (Figure 2). In addition to denitrification,

there are two other processes that contribute to nitrogen removal in wetlands: the incorporation of nitrogen into sediments by leaching and burial (sedimentation) and immobilization by plants, fungi and bacteria as part of nutrient uptake and storage during the growing season.

Denitrification is considered the main process of nitrogen removal and plays an important role in the biogeochemistry of wetlands (Saunders & Kalff, 2001). This process happens under anaerobic conditions mediated by heterotrophic bacteria that use nitrate (NO_3^-) or nitrite (NO_2^-) as a terminal electron acceptor and derive energy from the oxidation of organic matter to produce nitrogen gas (N_2) as a final product (Figure 3). Wetlands are considered the most efficient freshwater ecosystem for nitrogen removal, compared to lakes and rivers, because of the aerobic/anaerobic conditions that create suitable habitats for microorganisms to carry on the coupled biogeochemical processes of nitrogen cycle (Saunders & Kalff, 2001). However, when conditions are unsuitable for microorganisms to carry on complete denitrification reactions, incomplete denitrification can result in the production of noxious intermediaries gases such as NO, NO_2 and N_2O, can be produced (Knowles, 1982; Kampschreur et al., 2009).

Excessive production of N_2O in wetlands is of concern because N_2O is a greenhouse gas with a global warming potential 300 times than CO_2 also contribute to the destruction of stratospheric ozone (2011 IPCC guidelines). Studies in created wetlands have demonstrated that production of N_2O may be enhanced during the early stages of wetland establishment because of environmental conditions that limit the capacity for microorganisms to carry out complete denitrification (Hernandez & Mitsch 2006, 2007). In the case of highly impacted wetlands, an imbalance in the nitrogen cycle can also enhance the production of N_2O relative to N_2 compared

to processes that happen under natural wetlands, thereby impacting resilience, recycling and regulation of nutrients (Sirivedhin & Gray, 2006). For these reasons, there is a need to understand the role of nutrient availability (land use legacy), hydrology and carbon availability in created wetlands, how microbial communities may respond by contributing to less desirable outcomes (noxious gases) during early stages of development.

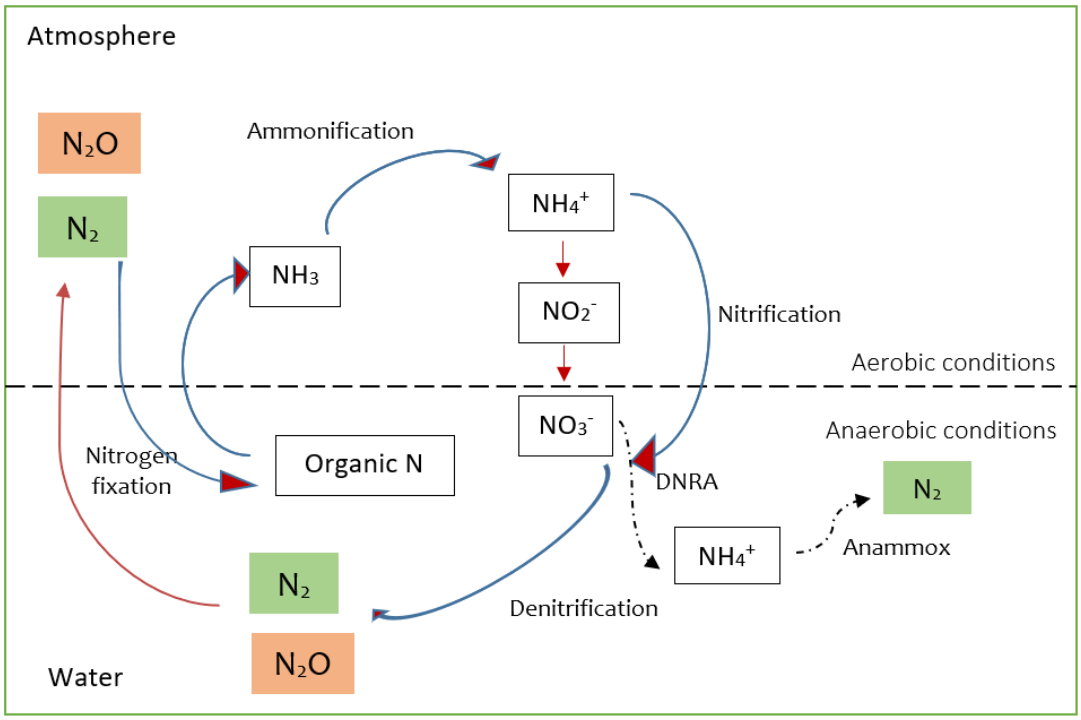

Figure 2. Nitrogen cycling pathways in wetlands

1.3 The influence of hydrology on denitrification

The hydrology of a wetland plays an important role in overall ecosystem function (Zedler, 1996), because it modifies soil properties and subsequently creates conditions for plants, animals and microorganisms to establish successfully (Morris, 1991; Calhoun et al., 2014). Thus, it impacts soil biogeochemistry and determines the biotic community structure (Mitsch & Gosselink, 2007;

Morris, 1991). Water budgets in wetlands are influenced by seasonal precipitation, evapotranspiration, groundwater inflow/outflow and surface inflow/outflow that fluctuate through time (Vepraskas et al., 2006). In some emergent wetlands (i.e. marshes, peatlands), surface inflow and groundwater are the main way sediments and nutrients enter the system.

The fluctuating water budget in wetlands determines oxygen availability in the soil and overlying water, thus determining the presence and relative extent of aerobic/anaerobic zones (Sirivedhin & Gray, 2006). As solutes diffuse across these redox boundaries microbial communities use those solutes to carry on several biogeochemical processes such as aerobic nitrification (Vilain et al., 2014) and anaerobic denitrification (Aulakh et al., 1991; Del Grosso et al., 2000; Estavillo et al., 1994; Mosier et al., 2002; Sirivedhin & Gray, 2006; Hunting & Geest, 2011). Recent studies have demonstrated that the constant changes in the hydrology of created wetlands are highly

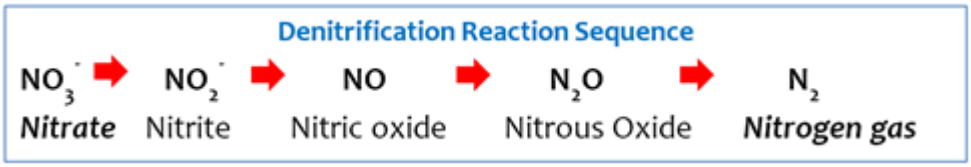

Figure 3. Schematic sequence of complete denitrification process

correlated to denitrification rates (Song et al., 2010), thus seasonal hydrology can have a major impact on N_2O production and consumption (Butterbach-Bahl et al., 2013) and because of the ongoing climate changes, it is crucial to understand the effects of extreme precipitation events in the early years of the wetland creation, particularly in created wetlands highly influenced by seasonal precipitation.

1.4 The influence of nitrogen availability on denitrification

The excessive use of fertilizers, confined livestock operations and the burning of fossil fuels has led to an accumulation of ammonium and nitrate in wetlands (Vitousek et al., 1997). Oftentimes, wetlands are created on lands that are reclaimed from agriculture, and as such have high levels of legacy nutrients in the soils. High background levels of nitrogen, coupled with additional input of nitrogen through surface inflow can exceed the wetlands' removal capacity, altering the biogeochemistry of the wetland (Smith et al., 1998), and thereby shifting the trajectory of development. It is fundamental to understand the response of biotically-controlled processes, such as denitrification, to the legacy of nutrients available when creating a wetland. For example, in the case of nitrate availability, it plays an important role predicting wetland behavior because it controls both plant and bacterial biomass production and microbial activity (Kadlec & Hammer, 1988).

Plant communities can also influence nitrogen availability in wetlands because plants are a sink and subsequent source for nitrogen. In natural wetlands, higher concentration of nitrogen in plants tissues is correlated to higher decomposition rates, subsequently impacting the concentration of soil organic carbon (%) and N (%), and consequently promoting denitrification and rates of nitrogen cycling (Fennessy et al., 2008). In created wetlands, plant diversity and structure may be limited because of the low nutrient availability (Fennessy et al., 2008), in addition decomposition rates are lower compared to natural ones coupled with poor movements of nutrients can lead to more anoxic conditions and negatively impact heterotrophic aerobic/anaerobic microbial communities activity and structure and vice versa (Hartman et al.,

2008), reducing nitrification/denitrification rates and creating a more unstable ecosystem that leads to incomplete nitrogen removal pathways (Schaafsma et al., 1999), which has a direct effect in the production of greenhouse gases released to the atmosphere (Meurer et al., 2016), limited denitrification also modifies soil chemistry (Schaafsma et al., 1999) leading to eutrophication and soil acidification (Smith et al., 1998) and ultimately shifting plant communities (Morris, 1991).

The direct effect of the availability of NO_3^- on denitrification rates (Knowles, 1982; Colbourn & Dowdell, 1984; Del Grosso et al., 2000; Sirivedhin & Gray, 2006; Morris, 2014) has been well studied in a variety of systems, including grasslands (Estavillo et al., 1994; Sánchez et al., 2001) and wetlands (Estavillo et al., 1994; Sirivedhin & Gray, 2006; Hernandez & Mitsch, 2007; Morris, 2014). When the concentration of nitrate is above or below a threshold for the system, it can inhibit denitrification acting as a control factor for denitrification rate (Tomaszek, 1995). However, it is also known that processes of the nitrogen cycle are also limited or controlled by other interactive factors such as soil moisture, dissolved oxygen and carbon availability (Sirivedhin & Gray, 2006). When these factors are not optimal in the system, final products of denitrification can also be affected, therefore the reduction of N_2O to N_2 can be inhibited (Colbourn & Dowdell, 1984) impacting the ratio of N_2O/N_2 and increasing the release of N_2O as a final product (Mosier et al., 2002).

Therefore, when creating a wetland, it is important to take in consideration the soil nitrogen availability to predict the response of microbial communities to carry on several biogeochemical processes, paired with potential management strategies to amend soil nutrients that can optimize positive responses and establishment of microbial communities, especially early

years of the creation process, to minimize the production of N_2O.

1.5 The influence of carbon availability on denitrification

Soil organic matter is another property that defines wetland functionality: highly organic soils release nutrients, resist soil compaction and have greater water holding capacity that benefits microbial and plant community establishment (Sutton-Grier et al., 2009). Restored wetlands with low organic matter and poor nutrient cycling may benefit from the addition of organic matter, which increases microbial activity and denitrification (Tomaszek 1995; Sirivedhin & Gray, 2006; Ballantine et al., 2011) by providing labile C to heterotrophic microorganisms (Del Grosso et al., 2000). To increase labile C in wetlands, research have been using straw, topsoil, biochar, sawdust, plant litter, glucose and activated carbon as soil amendments to stimulate nitrogen removal (Ballantine et al, 2014). Field studies in riparian wetlands have demonstrated that compost used as a source of carbon, increases denitrification (Sutton-Grier et al., 2009). Under laboratory conditions denitrification rates are highly correlated with the quantity and quality of organic matter added to the soil (Sirivedhin & Gray, 2006). Thus, the addition of organic matter as a management strategy may serve to promote beneficial effect on biogeochemical processes, including the enhancement of microbial communities and the removal of excess reactive nitrogen (Sutton-Grier et all, 2009). This is likely to be of greater importance early in the restoration/creation process prior to the establishment of mature plant communities (Dee & Ahn, 2012). Once plant communities are established, the type and diversity of plants is important because decaying plants add organic matter to the soil, enhancing denitrification by increasing

carbon availability (Song et al., 2010). Therefore, of particular interest are studies that will help understanding the interwoven effect of soil amendment to successfully promote plant and microbial community establishment in early years of the wetland creation. Further, it is crucial to better understand how initial site conditions and soil amendments strategies could increase the removal of reactive nitrogen without leading to enhanced greenhouse gas production.

1.6 Overview of study

The purpose of this study was to evaluate the role of experimental manipulation of carbon availability in two created wetlands that vary in hydrology and prior land use with a goal to develop recommendations for enhanced wetland restoration in the future. My main hypotheses were: 1) Large-scale carbon addition will have a positive impact on both potential denitrification and N_2O release and 2) Carbon availability interaction with nutrient availability and hydrology to determine the relative differences in potential denitrification and N_2O efflux across sites.

2. METHODS

2.1 Project Overview

To study the effect of hydrology, carbon availability and nitrogen availability on nitrogen removal and N_2O emissions from created wetlands, I utilized an ongoing experiment to evaluate the impact of municipal compost addition on plant communities and nitrogen cycling. I measured potential denitrification and *in situ* N_2O flux in two created wetlands that differ in legacy impacts of livestock grazing and hydroperiod, with and without the addition of organic carbon (compost). The wetlands also differ in their plant communities, which, while not manipulated as part of the experiment does vary between and within sites, may have an additional potential control on nitrogen cycling. N_2O fluxes were measured *in situ* using two types of chambers that isolated the impact of soil relative to soil and plant canopy influences. Nitrogen limitation of N_2O efflux and potential denitrification were measured in the laboratory. I also analyzed soil physicochemical properties (bulk density, organic matter content, soil water content, soil temperature, pH), soil nutrients (extractable and porewater nitrogen and phosphorus concentrations), hydrology (soil water content, precipitation), dissolved N_2O in porewater, and plant cover and community composition. The *in situ* measurements and site characterization were conducted every 4-6 weeks over the six month growing season in 2016. Porewater was collected in May and November 2016.

2.2 Study site

This study took place at the High Acres Nature Area (HANA), located in Monroe County, NY (Figure 4). Most of the original wetlands at HANA were drained as part of the Erie Canal

construction and agriculture development in 1820. HANA was purchased by Waste Management of New England and New York (WM) in 1986 and is currently open to the public year-round for passive recreation and as a conservation area. A series of wetlands was created in 2009 – 2012 as mitigation for expansion activities at the adjacent High Acres Landfill, and this study took place in two of these created wetlands (Cady Wetland [A2S] and the Packard Wetland Complex [A3A]).

The Cady Wetlands (Figure 4; 43°5'34.12"N, 77°22'45.02"W) were created in 2009 on a site previously used for row crop agriculture. The southern portion of the wetland used for this study is an emergent wetland and typically had permanent aboveground water year-round, influenced by rainfall and underground exchange in the growing season. However, an unusual drought in summer 2016 dried all standing water in July and August. The plant community was dominated by *Typha latifolia*, *Typha angustifolia* (broadleaf and narrowleaf cattail respectively), *Polygonum persicaria* (Smartweed) and *Lythrum salicaria* (purple loosestrife).

The Packard Wetland complex, located within the same management area, was created in 2012 (Figure 4; 43°5'13.34"N, 77°22'16.25"W) in a location that was historically used as a cow pasture. The experiment was conducted in wetland cell 3A, constructed as a wooded/shrub=scrub wetland. This wetland does not typically have standing surface water year-round, and its major water inflow is rainfall during the growing season. The plant community was more diverse than Wetland Cady, mainly dominated by *Phragmites australis* (common reed), *Daucus carota* (Queen Anne's Lace), *Solidago canadensis* (common Goldenrod), (common grass), *Polygonum persicaria* (Smartweed), *Alisma subcordatum* (water plantain), *Typha latifolia* and *Typha angustifolia* (broadleaf and narrowleaf cattail respectively).

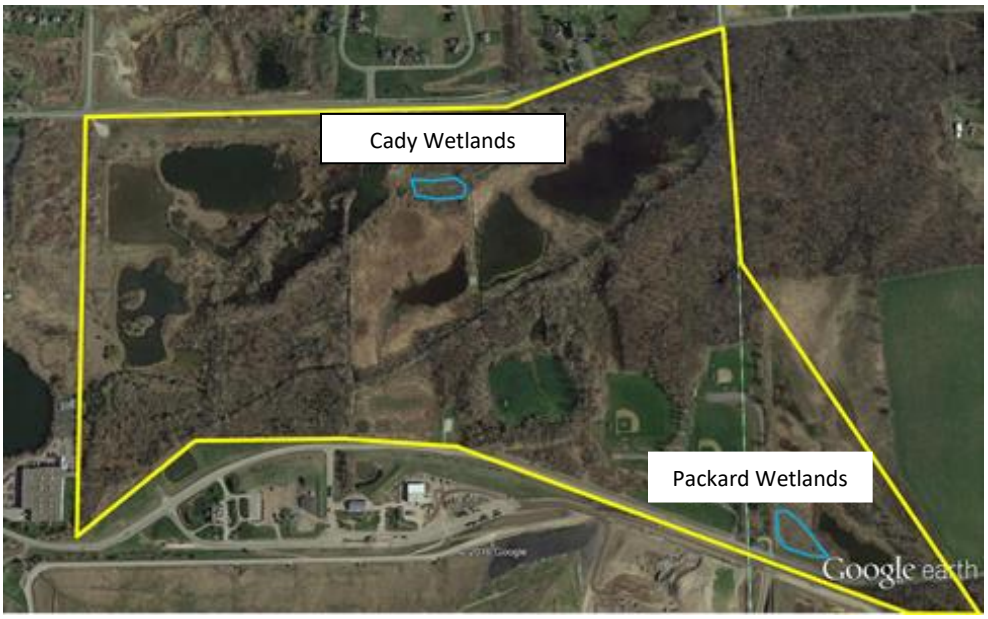

Figure 4. High Acres Nature Area (HANA) perimeter showing the location of both created wetlands for this study.

2.3 Experimental Design

In spring 2014, 10 experimental zones of 2 m * 30 m were installed in Cady Wetland to evaluate the impact of management practices on invasive species. In July 2014, organic matter (compost) was added to 5 of the 10 experimental zones (Figure 5) to a depth of approximately 5 cm. Compost was supplied by WM and consisted of degraded municipal leaf litter collected the previous fall and stored in managed compost piles at the nearby landfill. In May 2015 and June 2016, compost was added again to the same experimental zones. In spring 2015, 10 identical experimental zones were installed in the Packard Wetland and the same type of compost was added in 5 experimental zones in May 2015 and May 2016 (Figure 5). For the present study, 8 (4 pairs) of the 10 experimental zones installed at each site ((1,2), (3,4), (5,6) and (9,10)) were chosen for in-depth study based on a similar range of water depths (10-15 cm in Cady). In each

experimental zone, two 1 m^2 intensive sampling plots were installed for assessment of soil properties and soil gas exchange (n=8 per treatment) and one 0.36 m^2 intensive sampling plot was installed for measurement of ecosystem gas exchange (n=4 per treatment) in May 2016 (Figure 5).

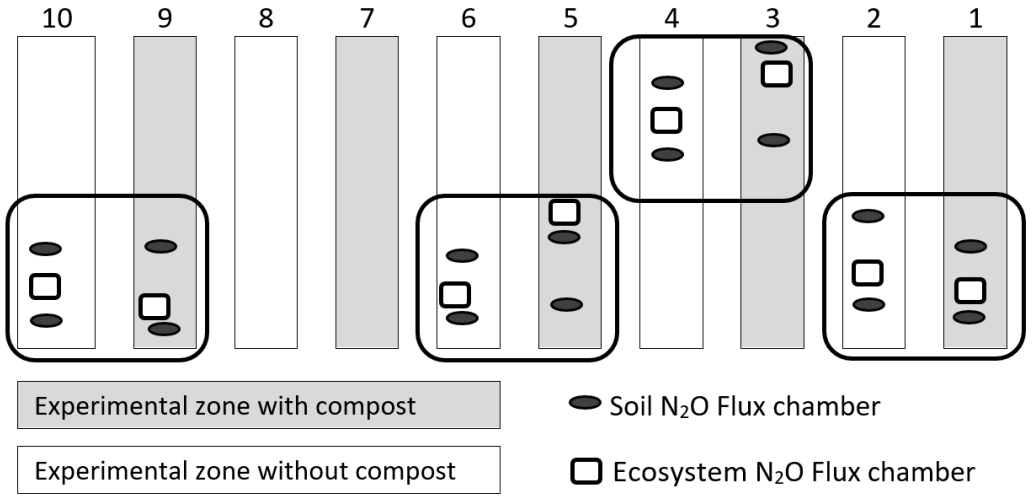

Figure 4. Project design showing 8 of the 10 experimental zones selected for this study, set of two soil N_2O flux chambers are inside of each intensive sampling plot of 1 m * 1 m and one square aluminum frame of a 0.6 m * 0.6 m was permanently installed to measure ecosystem N_2O flux.

2.4 Gas Flux and soil measurements

2.4.1 N_2O fluxes measurements in plants and soil

Fluxes of N_2O were measured *in situ* using the closed chamber technique in both small soil-only chambers and larger chambers that encompassed both vegetation and soil. Soil chambers were an adapted modification of Ryden & Skinner (1987) and consisted of two parts: a

polyvinylchloride (PVC) coupling or collar (0.115 m ID * 0.10 m length) inserted permanently at ground level in each of the two sampling plots per transect (Figure 6 (a)) and a polycarbonate tube (0.10 m ID * 0.0032 m wall * 0.30 m length) that was placed on the collar only during measurements (Figure 6 (b)). The chambers were sealed with a gas tight polycarbonate lid fitted with a butyl rubber O-ring. A subaseal stopper was inserted into a 15 mm hole in the lid. After the chamber was sealed, gas samples were taken every 10 min over a 30-minute period. The headspace in each chamber was mixed using a 60 ml syringe attached to Vincon tubing (0.0064 m ID* 0.1 m length) by drawing up 60 ml and pushing it back into the chamber three times prior to taking the final sample. Samples (10 ml) were taken with a 20 ml syringe (Figure 6 (c)) and either stored in the syringe until analysis within 24 hr or placed into an evacuated and flushed Exetainer. Soil temperature and air temperature were recorded. Chamber height was measured when gas samples were collected. The gas samples were analyzed using a gas chromatograph with an electron capture detector (Shimadzu GC-2014).

To measure *in situ* N_2O fluxes associated with both plants and soil, a bigger closed chamber was adapted to enclose the plant canopy (Figure 7). The objective of including plants in the chambers was to elucidate the role of plants in N_2O transport to the atmosphere through their aerenchyma. One chamber base constructed of a 0.6 m * 0.6 m square aluminum frame, was permanently inserted in each transect in June 2016 (Figure 5). The cover of the chamber (1.85 m H * 0.6 m W * 0.6 m D) was placed on the base only during measurements. The cover consisted of an aluminum frame enclosed with plastic greenhouse film on three sides (Figure 7), with the fourth side comprised of a polycarbonate sheet (0.32 cm). Attached to the polycarbonate sheet was a cooling system consisting of three small fans (12 cm*12 cm) to circulate the air inside the

chamber, and a heat exchanger connected by Tygon tubing to a submersible aquarium pump housed within a cooler containing ice water (Figure 7). Using a thermocouple OMEGA model HH802U, temperature inside and outside the chamber was measured continuously, and inside temperature was maintained between 21°C to 25°C by regulating flow through the cooling system. Inflow and outflow ports for CO_2 concentration measurements were also fixed to the outside of the chamber and connected to a portable LI-820 gas analyzer (concentrations were recorded at 2 second intervals). Gas samples were taken using a 20 ml syringe every 15 minutes for a 45 minute-period in the light and then the chamber was covered with Mylar sheeting and an additional 4 samples were taken at 15 minutes intervals. Gas samples were analyzed as described above. Plant composition and percent cover was assessed inside the chamber bases and the Shannon Weiner diversity index calculated in October 2016. Air temperature was also recorded

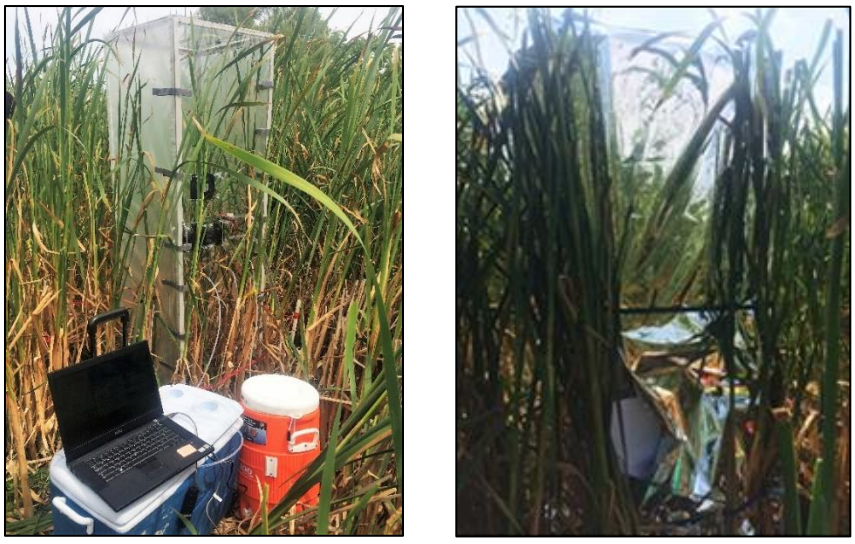

Figure 6: Chamber structure for measuring ecosystem N_2O gas fluxes (Under light [left] and dark conditions [right])

2.4.2 Dissolved N_2O in porewater

Cady Wetland has historically had standing water year-round, compared to Packard which dries completely in early summer. For this reason, one suction lysimeter was installed in each plot in wetland Cady (2 per experimental zone), porewater was collected and analyzed for porewater nutrients and dissolved N_2O. The suction lysimeter, or "sipper", was constructed of PVC tube (0.50 m L*0.04 m ID) with Teflon fritware on the bottom and a #9 two-hole stopper fitted with two stopcocks, one with a 0.5 m length of Vincon tubing extended to the bottom of the lysimeter (Chambers & Odum, 1990). Each lysimeter was inserted so the bottom of the pipe was 10 cm below the soil surface. Prior to sampling, each lysimeter was evacuated with N_2 gas to maintain an anaerobic environment and applied slight vacuum with a hand pump. Porewater samples (20 ml) were collected using a 60 ml syringe and immediately filtered in situ using a Supor syringe filter (0.45 um) into Whirl-pak bags (10 ml for Nitrate and Phosphate and 10 ml for ammonium) and stored at -20°C in the lab until analysis. A separate unfiltered porewater sample of 10 ml was collected for dissolved N_2O analysis within 24 hours. In the lab, 0.3 ml of H_3PO_4 and 10 ml of N_2 gas was added to the syringe containing the 10 ml porewater, samples were shaken for 1 minute, 5 ml of gas sample was transferred to a second syringe and then injected immediately to a gas chromatograph with an electron capture detector (Shimadzu GC-2014).

2.4.3 Potential Denitrification

Potential denitrification (PDNF) was measured using Acetylene Block technique (Ryden et al., 1987; Groffman et al., 2012) which assesses the activity of the enzymes present in soil when incubated under optimum anaerobic conditions. Acetylene blocks the reduction of N_2O to N_2,

causing the accumulation of N_2O, which is a more measurable gas by gas chromatography than N_2 because of its lower concentration in the atmosphere. Soil samples were collected from each plot using an auger (0.022 m diameter, 0.10 m depth), stored in Whirl-pak bags and transported on ice to the lab and analyzed within 24 hr. Ten g of sieved soil (#8 mesh, 2.38 mm opening) was placed into airtight 250 ml tall clear WM septa glass jar (S121-0250) containing 5 ml of media (nitrate 100 mg kg^{-1} + dextrose 40 mg kg^{-1} + chloramphenicol 10 mg kg^{-1}) and 5 ml of Nanopure water. The closed container was immediately purged three times with N_2 gas for 1 minute each, shaken for 30 seconds and then 25 ml of acetylene was added prior to shaking for one additional minute. Gas samples (5 ml) were taken at 0, 30, 60 and 120 min, glass jars were placed in the rotoshaker during incubation. Samples were analyzed immediately using a gas chromatograph with an electron capture detector (Shimadzu GC-2014).

2.4.4 Soil analytical methods

To assess the correlation of soil properties with PDNF and N_2O fluxes, two soil cores of 0.15 m depth per plot were collected using an auger (0.022 m ID), stored in whirl-pak bags, transported to the lab using a cooler to preserve physicochemical properties and stored at -20°C until analysis. Soil was collected every 4 to 6 weeks during the 6 month growing season.

Extractable inorganic nitrogen was measured on 5 g field-moist soil shaken with 2M KCL, centrifuged and the supernatant filtered (Keeney and Nelson 1982). Nitrate and Nitrite were measured with the cadmium reduction method using a Lachat Quickchem 8500 autoanalyzer (Lachat, 2003) and ammonium in the supernatant was analyzed with the phenol-hypochlorite method (Solórzano et al., 1969) using a Shimadzu 1800 spectrophotometer. To extract total

phosphorous, a subsample was mixed with 0.5 ml of 50% w/v $Mg(NO_3)_2$ and then combusted for 2 hr at 550°C, 10 ml of 1N HCl was added to the subsample prior analysis using the ammonium molybdate method (Murphy & Riley 1962). To measure inorganic phosphorous a similar procedure was followed but we omitted adding $Mg(NO_3)_2$ and combusting samples. The extracted samples were diluted 10:1 and analyzed using the ammonium molybdate method. All extractable nutrients were run in triplicate. Soil pH was measured by creating a 2:1 (v/v) slurry of deionized water to soil (Gelderman and Mallarino 2012) and measuring with a Hach pH probe calibrated with pH =4, 7, and 10 buffers.

The second soil core was used to analyze soil physical properties such as soil moisture, bulk density and soil organic matter. Soil moisture was measured gravimetrically after drying the soil at 60°C for 48 hours. Bulk density was measured using the air-dried soil weight (Blake & Hartge, 1986). The dried sample was used to determine percentage soil organic matter (SOM) by using loss on combustion method at 550°C for 4 hours (adapted method from Heiri et al., 2001).

2.4.5 Precipitation

Precipitation was a sum of ten days before soil collection, which happened the same days the soil chambers were evaluated. Precipitation data was obtained from weather history for Fairport, NY KNYFAIRP15.

2.5 Statistical analyses

Statistical analyses were conducted using JMP 15 Pro statistical software. Prior to analysis, Shapiro-Wilk's test was performed to check normality and homogeneity of variance. Values of

potential denitrification were converted to $\log_{10}$ to fit normality distribution. The experimental design allowed to analyze data using a one-, two- and three-way Analysis of variance (ANOVA) using site, treatment and month of evaluation as fixed factors, where appropriate. N_2O fluxes (soil and ecosystem), PDNF, and soil properties (nutrient availability, SOM, moisture content, precipitation, soil and air temperature, bulk density and soil pH) were analyzed using a full factorial three-way ANOVA with site (Cady, Packard), treatment (control, compost) and month of collection (May, July, August, September and November, when applicable) as fixed factors. One-way ANOVA was used to compare dissolved N_2O between treatments (control, compost) at Cady only. Two-way ANOVA was used to compare differences of plant cover and plant diversity between sites and treatments. For all ANOVAs, when significant difference was found ($p<0.05$) a Tukey's post-hoc test was applied to determine differences when there were more than two groups. T-test was used to find significant differences between N_2O fluxes under dark and light conditions.

Multiple regression was employed to determine the most important predictor variables for PDNF and soil N_2O fluxes. Variables used in the model included soil organic matter, soil moisture content, soil bulk density, soil pH, ammonium, nitrate, total phosphorus, soil temperature, air temperature and precipitation (calculated as the sum of the ten days before each analysis). One over-arching model was created for both sites, then each site was analyzed in a separate model. Akaike's Information Criterion (AIC) was used to select the best model based on the lowest AICc value (Anderson 2008).

3. RESULTS

3.1 N_2O gas fluxes

Soil N_2O fluxes measured in small chambers were highly variable for both sites, although significant differences between sites ($p<0.0001$*, Table 1; Figure 7a) and season were found ($p<0.0001$, Table 1). Overall, N_2O flux was higher in Cady than Packard (see Appendix B), the highest fluxes in the Cady wetland were observed in September in the compost treatment (compost: 9.8 $\pm$ 1.5 mg N_2O-N g m^{-2} d^{-1}). The highest flux in the Packard was also found in September but in the control treatment (control: 0.4 $\pm$ 0.0 mg N_2O-N g m^{-2} d^{1}). Despite differences within treatments, they were not significantly different ($p= 0.81$, Table 1). Ecosystem N_2O fluxes collected from the large chambers under light and dark conditions were only collected in August and October 2016. There were no significant differences between light and dark at either site in August (Cady: $p=0.70$; Packard: $p=0.71$, Appendix A) or October (Cady: $p=0.83$; Packard: $p=0.44$, Appendix A). Overall, Cady had higher N_2O fluxes than Packard in both months (Figure 7b).

3.2 Potential Denitrification

PDNF was similar at both sites ($p=0.20$, Table 1; Figure 7c). Although, differences between sites were influenced by the month of collection (site*month, $p<0.0001$, table 1). There was a significant effect of carbon addition on PDNF, which was also highly influenced by the month of collection ($p<0.0001$; $p<0.0001$ respectively, Table 1). There was a significant interaction between

site and month (p=0.0008), with the highest rates and substantial differences between treatments in the summer, but diminishing difference in the fall (Figure 7b).

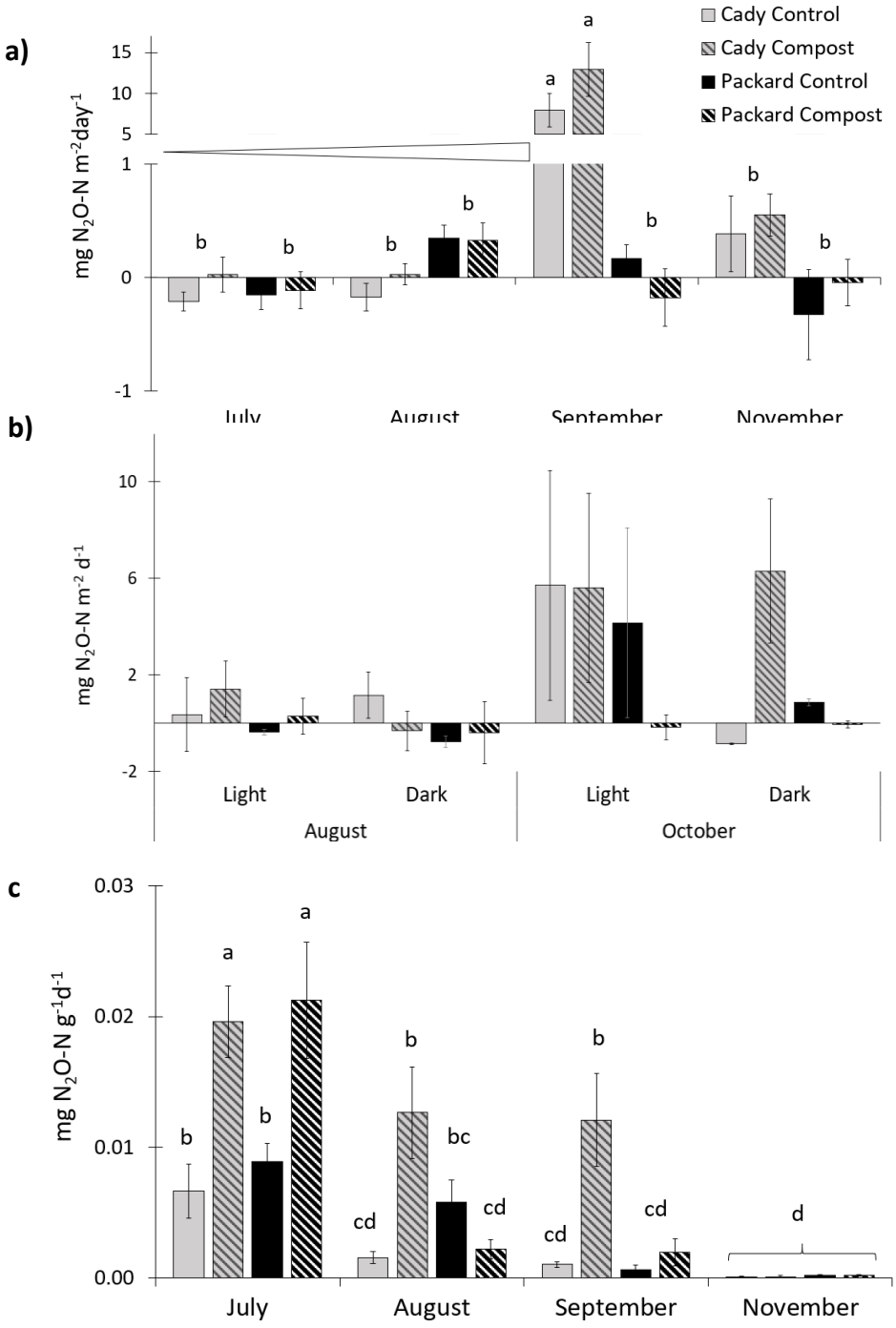

Figure 7: Soil N_2O fluxes (a), Ecosystem N_2O fluxes (b) and potential denitrification (c) in two created wetlands in summer/fall 2016. (Mean $\pm$ SE)

3.3 Porewater characterization

Lower precipitation and high temperatures during the spring/summer dried the standing water of Cady and reduced the water table of Packard by mid-summer; for this reason, porewater was only collected at site Cady June and November. Nitrate concentration in porewater was not influenced by compost addition (Table 3). Porewater phosphate concentration was slightly higher when compost was added, but not significantly so (Table 3). There was no significant difference in dissolved N_2O between treatments, but concentrations were significantly higher in November than in June ($F_{1,17}$= 0.1, p=0.81, $F_{1,17}$=17.8, p=<0.0009* respectively, Table 3).

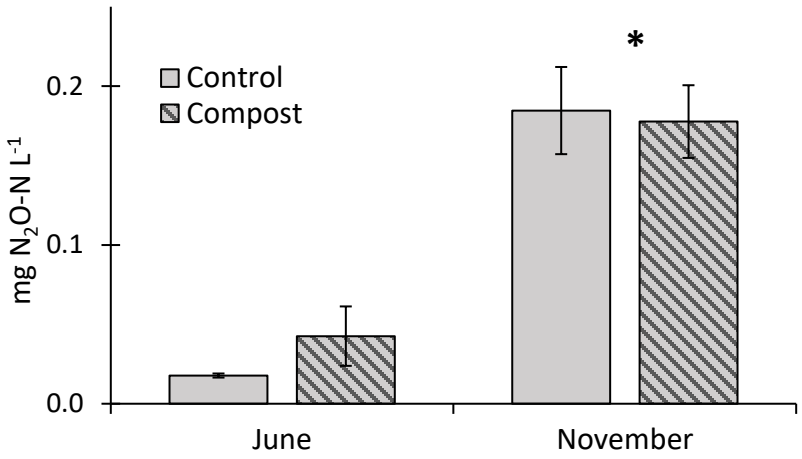

Figure 8: Dissolved N_2O (Mean $\pm$ SE) analyzed in porewater at site Cady. * indicates significant difference between month of collection.

Table 1: Results of ANOVA examining the effect of site (Cady, Packard), month (July, August, September and November when applicable) and treatment (control, compost) on soil flux, ecosystem flux and potential denitrification. Significant p-values are bolded and/or represented by an asterisk (*).

Factor	***Soil N_2O flux***		***Ecosystem flux***				***Potencial Denitrification***	
			Light		*Dark*			
	F	*p*	F	*p*	F	*p*	F	*p*
Site	$F_{1,116}$=35.2	*	$F_{1,22}$=1.2	0.30	$F_{1,20}$=0.4	0.55	$F_{1,84}$=1.7	0.20
Treatment	$F_{1,116}$=0.1	0.81	$F_{1,22}$=2.2	0.16	$F_{1,20}$=0.0	0.88	$F_{1,84}$=17.2	*
Month	$F_{3,116}$=39.6	*	$F_{1,22}$=0.9	0.35	$F_{1,20}$=0.4	0.56	$F_{3,82}$=43.7	*
*Site*Treat*	$F_{1,116}$=1.8	0.18	$F_{1,22}$=0.0	0.85	$F_{1,20}$=0.2	0.64	$F_{1,84}$=3.6	0.10
*Site*Month*	$F_{3,116}$=30.7	*	$F_{1,22}$=0.0	0.89	$F_{1,20}$=0.4	0.56	$F_{3,82}$=6.3	**0.001***
*Treat*Month*	$F_{3,116}$=0.0	1.00	$F_{1,22}$=0.0	0.55	$F_{1,20}$=0.7	0.43	$F_{3,82}$=3.0	**0.035***
*Site*Treat*Month*	$F_{3,116}$=0.8	0.80	$F_{3,82}$=3.0	0.99	$F_{3,82}$=3.0	0.13	$F_{3,82}$=0.6	0.60

3.4 Hydrology

Permanent standing water during spring/summer was the main distinctive characteristic of Cady compared to Packard, where the soil surface was dry during those seasons. However, low precipitation in the spring/summer of 2016 dried even the Cady Wetland, causing a complete disappearance of standing water for several weeks during the field measurements. Significant differences between sites and treatments were found ($p<0.0001$; $p=0.03$ respectively, Table 3), and were highly influenced by the month of soil collection ($p<0.0001$). Sites were significantly different in May, July and November (Table 2). Overall, the average of moisture content was 1.3 times higher in Cady than Packard (46.1 and 35.3 % respectively) and was increased by the addition of compost at both Cady (control: 42.9% and compost: 49.3%, table 3) and Packard (control: 33.1 and compost: 37.5%, Table 2).

The mean precipitation prior to the measurements was slightly higher in Packard than Cady (control and compost: 26.2 mm and control and compost: 22.4 mm respectively), suggesting that measurement processes happened in Packard more often after an event of rainfall compared to Cady, this difference was significant between sites and it was significantly impacted by the month of collection (site: $p=0.0244$; month: $p=<0.0001$; month*site: $p=<0.0001$).

3.5 Soil characterization and nutrient availability

SOM was higher in Cady than Packard in both control and treatment zones (Table 2) and increased significantly with addition of compost at both sites (control Cady =17.3$\pm$1.3 % and Packard =14.1$\pm$1.0 %; compost Cady = 19.2$\pm$1.8 % and Packard =17.2$\pm$1.2 %, Table 2). The

monthly mean SOM was higher for Cady than Packard (see appendix C and E), thus significant differences were found between sites ($p<0.0009$), treatments ($p<0.0025$) and month ($P=0.00462$, Table 3). Soil bulk density was higher in Packard than Cady (0.7 ± 0.1 and 0.6 ± 0.1 g/cm^3 respectively). Significant differences were found between sites, treatments and month of collection ($p=0.0007$, $p=0.0042$ and $p<0.0001$ respectively, table 3). Soil pH was significantly lower in Cady than Packard (7.3 ± 0.1 and 7.7 ± 0.1 respectively, appendix D) and significantly different between treatments ($p=0.0014$, Table 3). In Packard, higher pH was found in May and July (Table 2), differences between sites were also influenced by the month of collection ($p=0.0001$, Table 3).

Extractable ammonium was 2.5 times higher at Cady than Packard ($p<0.0001$; Table 2 and 3; appendix D) and influenced by month ($p<0.0001$). Despite lack of significance in the main effect of treatment ($p=0.11$), there was a significant three-way interaction (site*treatment*month $p= 0.0087$, Table 3). Nitrate was similar at both sites; however there is a significant effect of adding compost in the increase of nitrate availability in the soil ($p=0.02$, Table 3), that was time dependent ($p<0.0001$), leading to a site*month interaction (site*month, $p=0.0002$, Table 3).Total phosphorus was consistently higher in Packard than Cady in most of the soil collections, and it was higher in compost than control (control Cady = 86.7 ± 3.5 mg P/kg soil and Packard = 103.9 ± 5.3 mg P/kg soil ; compost Cady = 102.6 ± 5.4 mg P/kg soil and Packard = 110.5 ± 4.2 mg P/kg soil, Table 2), with significant differences between both sites and treatments ($p<0.0001$, $p<0.0001$ respectively; Table 3).

Table 2: Mean ± SE soil characterization measurements in Cady and Packard during spring/summer 2016.

Factor	Site	May		July		August	
		Control	*Compost*	*Control*	*Compost*	*Control*	*Compost*
Moisture content	*Cady*	77.8±0.1	73.5±0.1	35.2±0.0	55.4±0.0	27.1±0.0	27.5±0.0
(%)	*Packard*	52.1±0.1	63.1±0.1	21.8±0.0	21.9±0.0	22.7±0.0	24.7±0.0
Organic Matter	*Cady*	17.3±1.1	15.3±1.2	18.2±1.3	22.6±2.4	17.2±0.8	20.3±2.0
(%)	*Packard*	14.1±0.9	15.2±0.6	14.1±1.1	15.9±1.2	15.1±1.3	18.2±1.8
Bulk density	*Cady*	0.7±0.0	0.8±0.1	0.6±0.2	0.4±01	0.6±0.0	0.5±0.0
(g/cm^3)	*Packard*	0.8±0.1	0.8±0.0	0.7±0.1	0.7±0.0	0.6±0.1	0.6±0.1
pH	*Cady*	7.3±0.1	7.6±0.1	7.1±0.1	7.2±0.1	7.3±0.1	7.3±0.0
	Packard	7.9±0.1	8.0±0.1	8.1±0.1	8.2±0.0	7.3±0.1	7.3±0.0
Temperature	*Cady*	---	---	19.0±0.5	18.3±0.5	21.6±0.5	22.2±0.2
(°C)	*Packard*	---	---	19.2±0.4	18.9±0.4	22.1±0.4	22.4±0.4
Ammonium	*Cady*	57.8±6.9	36.9±6.2	16.1±4.0	34.1±7.3	21.6±3.9	18.4±2.2
(mg/kg)	*Packard*	9.9±1.6	9.6±2.1	6.7±2.0	3.7±0.8	14.2±1.8	13.9±0.9
Nitrate	*Cady*	0.2±0.2	0.3±0.2	55.5±6.0	80.5±6.0	66.1±11.7	88.1±10.9
(mg/kg)	*Packard*	18.2±3.2	26.3±3.4	40.4±6.3	50.5±5.8	62.6±8.2	67.8±4.3
Total P	*Cady*	80.2±5.7	95.3±9.7	84.7±2.8	106.9±5.8	86.0±5.4	91.4±3.2
(mg/kg)	*Packard*	105.9±7.5	105.9±3.6	91.8±4.4	108.5±2.5	107.4±2.7	114.6±5.9

Factor	Site	September		November	
		Compost	*Control*	*Compost*	*Compost*
Moisture content	*Cady*	34.0±0.0	35.2±0.0	58.3±0.0	62.7±0.0
(%)	*Packard*	27.4±0.0	29.7±0.0	41.7±0.0	48.4±0.0
Organic Matter	*Cady*	15.8±1.6	17.9±1.8	18.3±1.5	19.2±1.6
(%)	*Packard*	13.9±1.0	19.4±0.8	13.6±0.9	17.5±1.7
Bulk density	*Cady*	0.7±0.1	0.6±0.0	0.7±0.1	0.7±0.1
(g/cm^3)	*Packard*	0.7±0.0	0.5±0.0	0.8±0.1	0.7±0.0
pH	*Cady*	7.1±0.0	7.2±0.1	7.4±0.1	7.5±0.1
	Packard	7.4±0.1	7.5±0.0	7.6±0.1	7.8±0.1
Temperature	*Cady*	14.0±0.2	14.0±0.2	8.9±0.6	8.2±0.4
(°C)	*Packard*	15.6±0.0	15.4±0.0	5.4±0.3	5.4±0.3
Ammonium	*Cady*	39.5±2.9	68.1±7.1	28.3±4.6	38.5±2.6
(mg/kg)	*Packard*	38.8±3.6	33.2±1.9	6.2±0.4	6.1±0.4
Nitrate	*Cady*	19.5±5.1	15.3±4.1	27.1±5.0	24.5±6.0
(mg/kg)	*Packard*	15.7±2.6	19.4±4.4	32.8±3.8	37.1±4.2
Total P	*Cady*	101.4±2.6	123.7±6.0	81.0±1.1	95.7±2.3
(mg/kg)	*Packard*	118.0±8.7	121.2±6.8	92.56±3.2	102.2±2.3

3.7 Total plant cover and plant diversity index

At Cady, the sampled areas were mainly covered by *Phragmites australis* (common reed), *Typha latifolia* (broadleaf cattail) and *Typha angustifolia* (narrowleaf cattail), whereas the species at Packard were more indicative of wet meadow species: *Polygonum persicaria* (smartweed), *Epilobium parviflorum* (willow herb) and *Crepis* sp (hawk's beard). Total plant cover was higher in compost treatment than control (Figure 9) at both sites (Cady: control=13.7 and compost= 20.2; Packard: control=14.9 and compost 17.8) however there was no significant difference between sites ($F_{1,15}$=0.0, p=0.88) or treatments ($F_{1,15}$=1.3, p=0.27). Plant diversity was higher at Packard than Cady (H'=1.1 $\pm$ 0.3 and 0.8$\pm$0.2, respectively) but not significantly different ($F_{1,15}$=2.3, p=0.16) and there was no effect of compost addition ($F_{1,15}$=0.2, p=0.68).

3.8 Multiple regression models

Precipitation was a common negative predictor variable across all models for PDNF, along with the availability of nitrate/nitrite. In the combined model, soil pH and ammonium were also positively associated with PDNF. In the individual site models, soil moisture was a negative predictor along with ammonium at Cady, whereas at Packard, soil pH was a positive predictor. Across sites, ammonium, precipitation, and air temperature were positively correlated and nitrate/nitrite was negatively correlated with soil N_2O flux. At Cady, ammonium and nitrate remained in the model, but total phosphorus was also important. At Packard, soil temperature and pH were positively correlated and air temperature was negatively correlated with the N_2O flux.

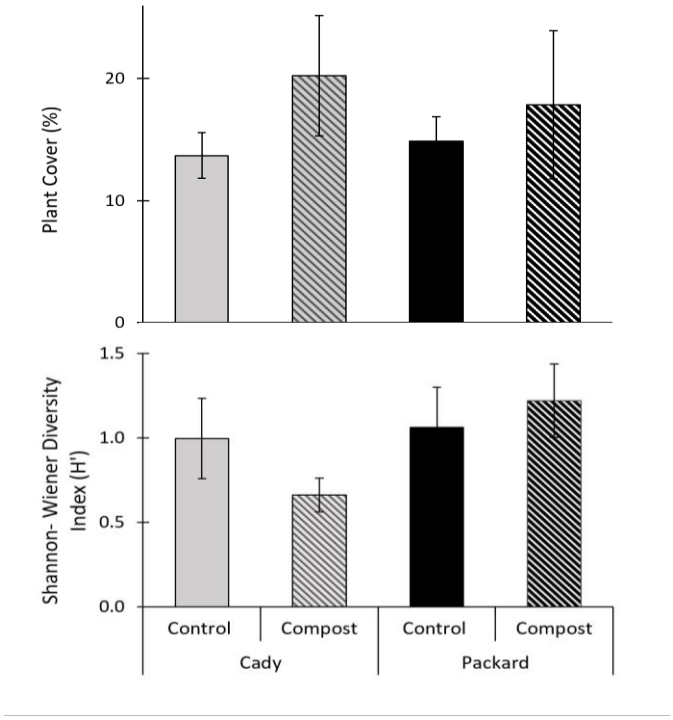

Figure 9: Plant cover and diversity index per treatment and sites evaluated at the end of September 2016.

Table 3: Results of ANOVA examining the effect of site (Cady, Packard), month of sample collection, and treatments (control, compost) on soil, soil characterization, soil nutrient availability and porewater nutrient availability. Significant p-values are bold.

Factor	***Site***		***Treatment***		***Month***		***Site*Treat***		***Site*Month***		***Treat*Month***		***Site*Treat* Month***	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Soil characterization														
Moisture content	$F_{1,154}$=53.3	*	$F_{1,154}$=5.1	**0.025***	$F_{4,151}$=77.2	*	$F_{1,154}$=0.1	0.82	$F_{4,151}$=5.0	**0.001***	$F_{4,151}$=1.1	0.37	$F_{4,151}$=2.8	**0.030***
Bulk density	$F_{1,158}$=11.9	**0.001***	$F_{1,158}$=8.5	**0.004***	$F_{4,155}$=13.2	*	$F_{1,158}$=0.0	0.98	$F_{4,155}$=1.7	0.16	$F_{4,155}$=1.1	0.36	$F_{4,155}$=1.1	0.36
pH	$F_{1,158}$=140.5	*	$F_{1,158}$=10.7	**0.001***	$F_{4,155}$=23.7	*	$F_{1,158}$=0.1	0.81	$F_{4,155}$=24.6	*	$F_{4,155}$=0.3	0.86	$F_{4,155}$=0.4	0.79
Precipitation	$F_{1,159}$=5.2	**0.024***	$F_{1,159}$=0.0	1.00	$F_{4,159}$=76.9	*	$F_{1,159}$=0.0	1.00	$F_{4,159}$=17.3	*	$F_{4,159}$=0.0	1.00	$F_{4,159}$=0.0	1.00
Temperature	$F_{1,108}$=4.6	**0.035***	$F_{1,108}$=0.8	0.36	$F_{4,105}$=985.8	*	$F_{1,105}$=0.4	0.53	$F_{4,105}$=27.0	*	$F_{4,105}$=0.6	0.60	$F_{4,105}$=0.5	0.93
Soil nutrient availability														
SOM	$F_{4,149}$=12.7	**0.001***	$F_{1,149}$=14.0	*	$F_{4,145}$=2.5	**0.046***	$F_{1,149}$=0.8	0.37	$F_{4,145}$=2.0	0.11	$F_{4,145}$=1.3	0.27	$F_{4,145}$=0.7	0.63
Ammonium	$F_{1,118}$=113.5	*	$F_{1,118}$=2.7	0.11	$F_{4,118}$=28.3	*	$F_{1,118}$=1.6	0.21	$F_{4,118}$=12.1	*	$F_{4,118}$=1.2	0.30	$F_{4,118}$=3.6	**0.009***
Nitrate/Nitrite	$F_{1,118}$=0.0	0.90	$F_{1,118}$=6.0	**0.016***	$F_{4,118}$=56.2	*	$F_{1,118}$=0.8	0.38	$F_{4,118}$=6.2	*	$F_{4,118}$=0.5	0.37	$F_{4,118}$=0.8	0.53
Total Phosphorous	$F_{1,149}$=27.4	*	$F_{1,149}$=21.9	*	$F_{4,146}$=10.0	*	$F_{1,149}$=3.8	0.05	$F_{4,146}$=2.0	0.11	$F_{4,146}$=0.9	0.45	$F_{4,146}$=0.6	0.68
Porewater characterization – Cady Wetland only														
Nitrate	---	---	$F_{1,32}$=0.1	0.77	$F_{1,32}$=1.2	0.31	---	---	---	---	$F_{1,32}$=2.0	0.16	---	---
Phosphate	---	---	$F_{1,46}$=1.9	0.18	$F_{1,46}$=1.7	0.19	---	---	---	---	$F_{1,46}$=2.0	0.15	---	---
Dissolved N_2O	---	---	$F_{1,17}$= 0.1	0.81	$F_{1,17}$=17.8	**<0.001***	---	---	---	---	$F_{1,17}$=0.2	0.66	---	---

Table 4: Multiple regression analysis with correlation coefficients, F values and *p* values for the effect of each significant predictor. Denitrification potential (PDNF) and soil N_2O flux models for an over-arching model across sites and for each site individually with AICc and R^2 values.

	Model	AICc	R^2	Variable	Coefficient	F	*p*
Combined	**PDNF**	151	0.66	*Precipitation*	-5.07	25.3	<0.0001*
				Nitrate/Nitrite	0.01	13.3	0.0005*
				Soil pH	0.47	5.8	0.0186*
				Ammonium	0.01	5.1	0.0281*
	Soil N_20	375	0.45	*Ammonium*	0.10	10.3	*0.0022**
				Precipitation	1.56	4.8	*0.0332**
				Nitrate/Nitrite	-0.05	4.5	*0.0378**
				Air Temperature	0.27	4.3	*0.0425**
Cady (A2S)	**PDNF**	72	0.62	*Nitrate/Nitrite*	0.01	7.6	0.0112*
				Soil moisture content	-3.66	7.6	0.0115*
				Ammonium	0.02	5.2	0.0323*
				Precipitation	-1.06	4.8	0.0309*
	Soil N_20	214	0.65	*Ammonium*	0.12	5.6	*0.0256**
				Nitrate/Nitrite	-0.07	5.6	*0.0266**
				Total Phosphorus	0.10	5.5	*0.0279**
Packard (A3A)	**PDNF**	90	0.77	*Precipitation*	-0.51	13.9	0.0006*
				Nitrate/Nitrite	0.02	8.1	0.0072*
				Soil pH	0.51	4.6	0.0394*
	Soil N_20	102	0.40	*Soil Temperature*	0.16	7.7	*0.0087**
				Soil pH	1.01	6.1	*0.0184**
				Air Temperature	-0.13	5.2	*0.0281**

4. DISCUSSION

The unique characteristics of individual creation sites complicate using a one-size-fits-all approach to management of wetlands (Zedler, 2003). In this study, we found that created wetlands that differ in hydrology and prior land use behaved differently throughout the growing season, particularly influenced by seasonal variation in temperature and precipitation. Hence, it is important to understand how management techniques can favor specific ecosystem functions (i.e., nitrogen removal), and limit undesirable functions (i.e., N_2O release) when creating a wetland, especially under ongoing climate change.

Soil amendment is a management technique that increases soil organic matter content and improves soil properties (Sutton-Grier, 2009) influencing plant and microbial communities, and thus impacting biogeochemical cycling in wetlands (Ballantine et al., 2014). When adding carbon to the soil, it stimulates a synchronization of nutrient availability for plant and microbial communities (Fontaine et al 2007), ultimately playing a key role in the coupling of carbon and nitrogen biogeochemical cycling, i.e. denitrification (Gruber & Galloway, 2008, Song et. al 2014). In this study, we found that carbon addition had a significant positive influence on physical properties of soil - organic matter, moisture content, pH, bulk density and nutrient availability - confirming results of prior work elsewhere (Ballantine et al., 2014; Ruehlmann & Körschens, 2009; Sutton-Grier et al., 2009). Thus, the studied sites differed significantly in soil properties under control (no amendment) conditions. Overall, Cady had higher concentration of organic matter and ammonium than Packard confirming the more anaerobic conditions presence at Cady (Austin et. al. 2019), but similar nitrate concentration between sites was found. In addition, soil

moisture, bulk density and pH were also higher in Cady than Packard suggesting that differences between sites may be related to the interaction between prior use, hydrology land and current wetland structure.

Carbon control of biogeochemical processes such as denitrification has been previously demonstrated (Ballantine et al., 2014), and we found a similar positive impact of carbon addition on PDNF. Denitrification rates were higher in Cady than Packard, and were directly influenced by nitrate and ammonium availability, soil moisture content and precipitation (Sirivedhin & Gray, 2006). Moreover, both sites had more or less similar nitrate availability (>12.0 mg N g $soil^{-1}$), suggesting there was enough substrate for microorganisms to carry on denitrification across seasons (Del Grosso et al., 2000). However, the low rainfall during summer/fall season confirms the key role of SOM, the effect of carbon addition on denitrification can trigger higher removal of reactive nitrate from wetlands (Sutton-Grier et al., 2009) and nitrogen removal is one of the ecosystem functions driving the increased effort to create wetlands (Zhang et al., 2010). However, higher denitrification rates could also lead to higher greenhouse N_2O emissions from immature or hydrologically variable systems like created wetlands, especially in early stages of ecosystem development when the system is more sensitive to perturbation.

N_2O fluxes (soil and ecosystem) were not affected by adding carbon to the soil, although soil N_2O fluxes were higher in Cady than Packard in some months of the study, and were highly influenced by seasonal hydrology (Song et al., 2010). In addition, spatial variability found in these wetlands when measuring N_2O fluxes (Butterbach-Bahl et al., 2013) prevented the identification of environmental patterns. N_2O fluxes obtained using the ecosystem chambers were lower than

the fluxes from soil, suggesting little influence of the plants in transporting or storing N_2O.

The hydrology defines local wetland structure and the synergistic interaction with other factors, especially under different climate scenarios, is paramount. In this study we found that higher N_2O fluxes were found after a rainfall event, suggesting the regulatory influence of hydrology in N_2O production (Del Grosso et al., 2000; Hernandez & Mitsch, 2007; Vilain et al., 2014), especially in systems with a water budget highly dependable on precipitation (Morris, 2014). However, temperatures below 5°C seem to generally cease N_2O production (Morris, 1991), and we observed that temperature also acted as a controlling factor for N_2O fluxes, with low N_2O production during colder months, in spite of waterlogged conditions.

In addition, Cady seemed to have more microbial activity when soil moisture content reached an optimal threshold (Song et al., 2010), especially when nitrogen availability was higher. Although, these conditions also led to higher N_2O production, suggesting that incomplete denitrification was happening or there was high nitrification. Studies in nitrification have shown that when dissolved oxygen is insufficient, nitrifying microbes may use NO_2^- as a terminal electron acceptor and release N_2O as a final product (Kampschreur et al., 2009). In addition, ammonium concentration was higher in Cady than Packard, especially in September after a rainfall event. The highest concentration of ammonium (68.1 mg NH_4^+-N mg/kg soil) seems to be related to the highest soil N_2O emissions found in Cady in soil with carbon addition, indicating that carbon and ammonium interact to control N_2O production (Morris, 1991). Higher ammonium availability is also associated with higher nitrous oxide emissions under aerobic, sub-aerobic and anaerobic conditions (Huang et al., 2014). Studies have shown that the major sink for ammonium is plant

uptake (Morris, 1991), which may explain the different concentration of ammonium between sites, where the more diverse plant community at Packard may decrease available nutrients in the soil. Although, when comparing plant cover and diversity between sites, there were no significant differences. The higher availability of ammonium at Cady may also be driven by interplaying factors -high concentration of carbon and nitrate- that favors dissimilatory nitrate reduction to ammonia (DNRA) over denitrification, especially after rainfall, which may also be driven by the more saturated and thereby anaerobic soils. There is also a relationship between soil pH and ammonium, as it was found by Morris (1991), higher concentration of ammonium can lead to changes in pH.

Total phosphorus was less variable than nitrate and ammonium at both sites. Phosphorus availability in created wetlands is correlated to hydrology (Mitsch et al., 1995); high flow wetlands tend to remove more phosphorus than low flow wetlands. However, unusual drought conditions of the growing season in 2016 may have resulted in the relatively high total phosphorus concentration we observed (> 80 mg P/kg soil). Phosphorus was higher at Packard, likely as a legacy of prior land use. Total phosphorus was higher in soil with compost in site Cady, which ultimately may have impacted denitrification, as illustrated elsewhere (Kim et al., 2015) and in our regression model. The highest rate of PDNF was observed at Cady when phosphorus was also highest, illustrating a potential regulatory effect of phosphorus on heterotrophic processes and denitrification in particular because phosphorus plays an important role in the regulation of labile carbon availability for microbial activity and thereby key processes such as denitrification (Kim et al., 2015).

5. CONCLUSIONS

This study demonstrated that adding compost addition, as a management technique improves soil quality and favor denitrification rates, therefore it has a positive effect on removal of reactive nitrogen from soil but had no effect on N_2O emissions. This suggests a positive remediation of ecosystem function in the presence of compost, without concomitant increases in greenhouse gas production. N_2O fluxes from both soil alone and ecosystem-level were highly variable with few patterns, but generally higher after a rainfall event. Therefore, interannual patterns of precipitation are key drivers of overall N_2O emissions in created wetlands with a water table dependable in precipitation. Standing water plays an important role limiting diffusion of N_2O to the atmosphere, and overall moisture content influences coupling of nitrification and denitrification.

The synergistic effect of nutrient availability (nitrate and ammonium), precipitation and soil pH found in this study are key drivers of denitrification and N_2O production. Therefore, finding a concentration threshold of nutrient availability that supports complete denitrification while still limiting N_2O emissions could be a good management strategy. This study provides important data for unusual climate conditions that should be taken in consideration when creating a wetland, hence the importance of controlling interplaying factors in created wetlands to reduce their susceptibility to ongoing climate change.

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APPENDICES

Appendix A: Mean ± SE ecosystem fluxes measured under light and dark conditions

Factor	August		October		T-test (p value)	
	Control	Compost	Control	Compost	August	October
Cady						
Ecosystem N_2O flux *(mg N_2O-N g m^{-2} d^{-1})*					***0.70***	***0.83***
Light	0.4±1.5	1.4±1.2	5.7±4.8	5.6±3.9		
Dark	1.2±0.9	-0.3±0.8	-0.9±0.0	6.3±3.0		
Packard						
Ecosystem N_2O flux *(mg N_2O-N g m^{-2} d^{-1})*					***0.71***	***0.44***
Light	-0.4±0.1	0.3±0.8	4.2±3.9	-0.2±0.5		
Dark	-0.8±0.2	-0.4±1.3	0.9±0.1	-0.1±0.2		

Appendix B: Mean ± SE fluxes and denitrification per treatment measured throughout the study period.

	Control		Compost	
Factor	Cady	Packard	Cady	Packard
Soil N_2O flux *(mg N_2O-N g m^{-2} d^{-1})*	1.9±0.6	0.1±0.2	2.6±0.5	0.0±0.2
Ecosystem N_2O flux *(mg N_2O-N g m^{-2} d^{-1})*				
Light	3.0±3.2	1.9±2.0	3.5±2.5	0.1±0.6
Dark	0.2±0.5	0.0±0.2	3.0±1.9	-0.2±0.7
Potential DNF *(mg N_2O g^{-1} d^{-1})*	0.002±0.001	0.004±0.001	0.011±0.002	0.006±0.002

Appendix C: Mean ± SE Porewater chemistry measured in Cady in three months in 2016.

	May		June		November	
Porewater	Control	Compost	Control	Compost	Control	Compost
Phosphate *(mg/L)*	0.009±0.004	0.042±0.011	0.026±0.011	0.041±0.020	0.019±0.005	0.008±0.003
Nitrate *(mg/L)*	0.007±0.002	0.010±0.002	0.011±0.003	0.021±0.006	0.016±0.004	0.014±0.004

Appendix D: Mean ± SE soil physicochemical properties measured in Cady and Packard throughout the study period.

Factor	Cady		Packard		Site		Treatment		Site*Treatment	
	Control	Compost	Control	Compost	F	*p*	F	*p*	F	*p*
Soil moisture content (%)	46.5±0.0	50.9±0.0	33.1±0.0	37.5±0.0	1,154=53.3	**<0.0001**	1,154=5.1	**0.0251**	1,154=0.1	0.82
Organic Matter (%)	17.3±1.3	19.2±1.8	14.1±1.0	17.2±1.2	4,149=12.7	**0.0005**	1,149=14.0	**0.0003**	1,149=0.8	0.37
Bulk density *(g/cm^3)*	0.6±0.0	0.6±0.1	0.7±0.1	0.7±0.0	1,158=11.9	**0.0007**	1,158=8.5	**0.0042**	1,158=0.0	0.98
pH	7.2±0.1	7.3±0.1	7.7±0.1	7.7±0.1	1,158=140.5	**<0.0001**	1,158=10.7	**0.0014**	1,158=0.1	0.81
Precipitation (mm)	22.4±0.6	22.4±0.6	26.2±4.2	26.2±4.2	1,159=5.2	**0.0244***	1,159=0.0	1.00	1,159=0.0	1.00
Soil Temperature*(°C)*	15.9±0.4	15.7±0.4	15.6±0.3	15.5±0.3	**1,108=4.6**	**0.0350**	**1,108=0.8**	0.36	1,105=0.4	0.53
Ammonium *(mg/kg)*	32.7±4.4	39.2±5.1	15.1±1.9	13.3±1.2	**1,136=123.5**	**<0.0001**	**1,136=1.4**	0.23	1,136=4.6	**0.0343**
Nitrate *(mg/kg)*	33.7±5.6	41.7±4.8	33.9±5.4	40.2±4.4	**1,136=3.4**	0.07	**1,136=6.7**	**0.0111**	1,136=1.1	0.29
Total P *(mg/kg)*	86.7±3.5	102.6±5.4	103.9±5.3	110.5±4.2	1,149=27.4	**<0.0001**	1,149=21.9	**<0.0001**	1,149=3.8	0.05